NKp30 Enables NK Cells to Act Naturally with Fungi

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NK cells have direct activity against fungal pathogens. Using an unbiased systematic approach, Li et al. (2013) find that NKp30 is a major NK cell receptor responsible for fungal recognition. Moreover, diminished NKp30 expression is associated with reduced antifungal activity in NK cells isolated from HIV-infected persons.

Natural killer (NK) cells are cytotoxic lymphocytes, which are important effector cells in viral infections and anti-tumor immunity. Unlike cells of the adaptive immune system (T and B lymphocytes), there is no somatic receptor gene rearrangement in NK cells. NK cell receptors are germ-line encoded proteins and comprise several transmembrane inhibitory and activating molecules that regulate NK cell function and homeostasis. Within this group are killer-cell-Ig-like-receptors (KIRs), lectin type receptors, and natural cytotoxicity receptors (NCRs). The effector functions of NK cells are determined by a fine balance of signals received from these and other receptors, including the innate immune Toll-like receptors. The identification and cloning of three NCRs, NKp46, NKp30, and NKp44, were achieved in the late 1990s and revealed that NKp46 and NKp30 are expressed by NK cells freshly purified from peripheral blood, whereas NKp44 is induced upon NK cell activation (Hudspeth et al., 2013).

In addition to their central role in defending against tumors and infectious agents, NK cells have direct activity against human bacterial, parasitic, and fungal pathogens. Regarding the latter, activity has best been studied with *Cryptococcus neoformans*, an opportunistic fungus that causes an estimated million cases of meningitis in AIDS patients annually (Park et al., 2009). NK activity requires signaling via the PI3K-ERK1/2 signaling pathway and is mainly mediated by the lytic protein perforin (Wiseman et al., 2007). Similar direct interactions of NK cells against numerous other human fungal pathogens have been described, including *Candida albicans*. Moreover, whereas several studies have suggested NK cell recognition of fungi is a receptor-mediated process, specific receptors heretofore have not been identified (Jones et al., 2009; Levitz et al., 1995).

In the study reported in this issue, Li and colleagues used an unbiased systematic approach to identify receptors used by NK cells for fungal recognition and microbicidal activity (Li et al., 2013). The authors generated a panel of mouse monoclonal antibodies to human NK cells and found that one of the antibodies blocked NK cell binding to *Cryptococcus*. Further studies established that the antibody recognized the NK-activating receptor NKp30 and that NKp30 is necessary for optimal NK cell recognition, microbial synapse formation, and antimicrobial activity against *C. neoformans* and *C. albicans* (Figure 1). Additionally, NKp30 was shown to be required for perforin degranulation and signaling via PI3K/Akt and ERK phosphorylation in response to fungal stimulation. Importantly, given the strong association of cryptococcosis with AIDS, Li et al. (2013) demonstrate that NK cells from HIV-infected persons have impaired NKp30 expression, defective fungus-induced perforin release, and impaired killing of *C. neoformans*. Finally, they establish the proof-of-concept that these defects can be reversed: in vitro treatment of NK cells from HIV-infected persons with the proinflammatory cytokine IL-12, which has been shown to enhance the antifungal activity of these cells, restores their NKp30 expression and fungicidal capacity.

These findings have important conceptual and practical implications that extend beyond the identification of the first NK cell receptor that mediates direct recognition of fungi. The innate immune system recognizes fungi via a diverse array of receptors, including C-type lectin receptors, Toll-like receptors, scavenger receptors, and Fc/complement receptors. The findings by Li et al. (2013) suggest that the natural cytotoxicity receptor NKp30 should be added to the list of pattern recognition receptors for fungi. Translationally, the observation that the defective in vitro antifungal activity of NK cells from persons with HIV can be reversed with IL-12 suggests avenues for treatment of cryptococcosis in HIV-infected patients. Unfortunately, the adverse effects of IL-12 administration probably preclude its use in patients. More promising perhaps is IFNγ, which has been effectively used as an adjunctive immunotherapy for patients with cryptococcosis and has been shown to restore deficient fungus-stimulated IL-12 responses from HIV-positive mononuclear cells.

This study represents an important step toward understanding the mechanisms by which NK cells contribute to antifungal immunity, yet important questions remain. Upon IL-12 treatment, NKp30 expression is upregulated but the pathway leading to NKp30 induction downstream of IL-12 exposure and the mechanism mediating the recovery of the associated fungal cytotoxicity remain to be characterized, including whether the IL-12-augmented response is perforin dependent. NKp30 expression has also been shown to increase in HCV-exposed

but uninfected intravenous drug users (Golden-Mason et al., 2010) and in response to the cytokine IL-2 (Castriconi et al., 2003), suggesting that expression of the receptor can be regulated. However, it is unknown whether this regulation is directly mediated by IL-12 or through an intermediary such as IFNγ.

The study by Li et al. (2013) was performed in vitro using a human NK cell line and ex vivo using NK cells obtained from human blood. It remains an open question how important NK cells are for defenses against fungal infections in vivo. In murine models, while NK cell depletion or dysfunction has generally resulted in increased susceptibility to experimental fungal infections, in some studies deleterious effects have not been seen. Second, a relatively high effector-to-target ratio is generally required for in vitro antifungal activity yet only about 15% of circulating human lymphocytes are NK cells. Finally, while NK cells are dysfunctional in HIV-infected individuals, it may be a leap of faith to link NK cell dysfunction to susceptibility to *Cryptococcus* and *Candida* given the predominant role of CD4+ T cells. Similarly, primary hemophagocytic lymphohistiocytosis, a rare hematologic disorder of infancy with many genetic causes, is associated with both defective NK cell cytotoxicity and increased susceptibility to mycoses, but it is not clear if the association is causal given the multiple other immunocompromises that patients with this disease have.

Remarkably, Li et al. (2013) found that NKp30 is a receptor for two diverse fungal species; *C. neoformans*, a basidiomycete, and *C. albicans,* an ascomycete. While the fungal ligand recognized by NKp30 is unknown, it is likely to be a very well-conserved fungal "pathogen-associated molecular pattern" (PAMP) when one considers that *Cryptococcus* and *Candida* are members of phyla thought to have diverged at least 400 million years ago. Moreover, NKp30 is "promiscuous" in the sense that it recognizes viruses and parasites in addition to fungi, suggesting that the PAMP is more broadly shared among microorganisms. NKp30 is required for NK-mediated activity against both encapsulated and acapsular strains of *C. neoformans*. Li et al. (2013) speculate that the ligand recognized by NKp30 lies in the cell wall rather than being a capsular antigen. If this is the case, it remains to be explained how NKp30 would reach this ligand in encapsulated *C. neoformans*. Interestingly, Joyce et al. (2011) recently described that NKp30 displays an I-type Ig-like topology consisting of eight β strands forming two antiparallel β sheets. In *Drosophila*, the structure of GNBP3, a pattern recognition receptor capable of binding the fungal cell wall, consists of two antiparallel β sheets and belongs to the immunoglobulin fold family. This receptor has been described to specifically recognize long β-1,3-glucan chains, an important component of the fungal cell wall and a very well conserved PAMP among fungal species (Mishima et al., 2009). Finally, it should be noted that NKp30 is unlikely to be the sole NK receptor involved in fungal recognition as inhibition of NKp30 with monoclonal antibodies or knockdown with siRNA only partially inhibited conjugate formation and perforin release. In fact, Li et al. (2013) raise the possibility that a multistep process is involved in binding and antifungal effector activity of NK cells and that additional NK receptors are involved.

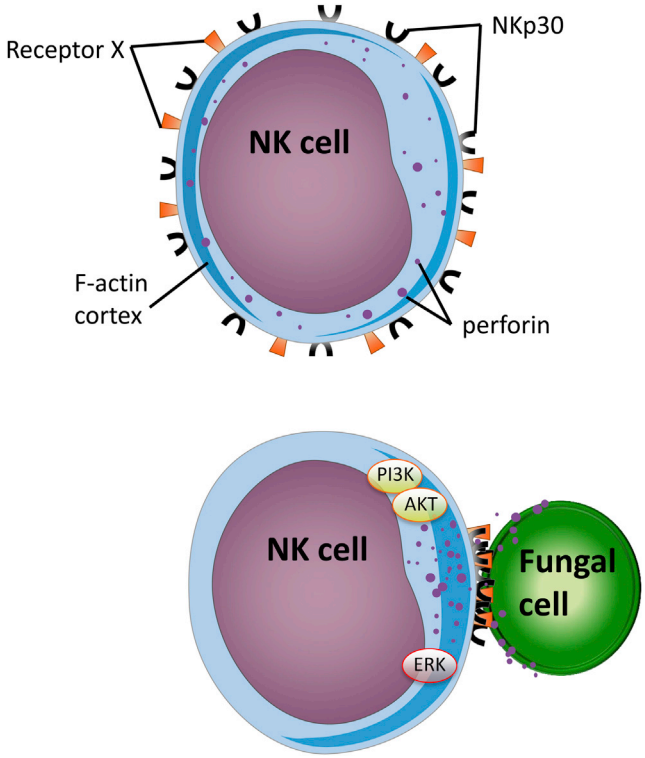

Figure 1. Overview of the Mechanism by which NK Cells Recognize Fungi and Mediate Antimicrobial Activity

Upon an encounter with a fungal human pathogen, NKp30 and possibly other receptor(s) (Receptor X) on NK cells recognize unknown fungal cell wall ligand(s). Recognition triggers signaling via the PI3K/AKT and ERK pathways, which leads to the formation of a microbial synapse between the NK cell and the pathogen. NKp30 and other receptor(s) polarize to the microbial synapse together with perforin and F-actin. Lytic degranulation then delivers effector molecules, such as perforin, to the fungal cell.

Despite these unanswered questions, the study reported by Li and colleagues is a major step in understanding the direct role of NK cells in antifungal immunity and describes a receptor on NK cells mediating recognition and antimicrobial activity of *C. neoformans* and *C. albicans*. Hopefully, these data will prompt research aimed at further deciphering these questions and designing novel approaches for the therapy of HIV-associated fungal infection.

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Dynamins Are Forever: MxB Inhibits HIV-1

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Human MxA (MX1) protein is an interferon-induced restriction factor for a diverse range of viruses, whereas the related MxB (MX2) protein was thought to lack such activity. Three recent papers, including one in this issue of *Cell Host & Microbe*, show that MxB inhibits human immunodeficiency virus type 1 (HIV-1) infection.

Myxovirus resistance (Mx) proteins are IFN-induced dynamin-like large GTPases of vertebrates (Haller and Kochs (2011). Most mammals have two Mx genes (myxovirus resistance genes 1 and 2) that arose by gene duplication. The human MxA protein (encoded by the MX1 gene) has long been recognized as a potent cell-autonomous restriction factor with antiviral activity against a range of pathogenic DNA and RNA viruses, notably influenza A viruses (Mänz et al., 2013). In contrast, MxB (encoded by the MX2 gene and closely linked to MX1 on chromosome 21) was found to be devoid of antiviral activity and was considered to serve cellular functions, such as regulating nucleo-cytoplasmic transport and cell-cycle progression (King et al., 2004; Melén et al., 1996). Liu et al. (2013) now report in this issue of *Cell Host & Microbe* that MxB (MX2) is an innate immunity factor that blocks HIV-1 infection. Two other recent reports published in Nature (Goujon et al., 2013; Kane et al., 2013) come to the same conclusion. These unexpected findings represent a major advance in our understanding of antiretroviral host defense and may provide clues for designing new strategies to combat HIV-1 and AIDS.

The human MX genes were cloned at the University of Zürich, Switzerland, almost 25 years ago (Aebi et al., 1989), at a time when the AIDS epidemic was gaining momentum worldwide. How is it possible that the anti-HIV-1 activity of MxB remained undetected for such a long time? MxB was perhaps neglected owing to the preconceived notion that it was not antiviral or it was outright missed because it displays its antiviral function preferentially in some cell types but not others. In retrospect, it makes sense that MxB is part of the IFN-induced antiviral state, as it was sporadically identified in large scale screens for IFN-induced antiviral factors.

MxA and MxB are closely related (63% amino acid sequence identity) and share a similar domain structure and architecture (Figures 1A and 1B). The crystal structure of MxA revealed three functional domains, namely the amino-terminal globular "G domain" that binds and hydrolyses GTP, a hinge-like "bundle signaling element" (BSE) that connects the "G domain" to the elongated "stalk" domain, and the helical "stalk," which mediates self-assembly into oligomers and serves antiviral effector functions (Gao et al., 2011). The predicted structure of MxB is almost superimposable with that of MxA (Figure 1B). A unique feature of MxB is that it exists in two isoforms that are translated as 78 or 76 kDa proteins from alternate AUG start codons of the same mRNA (Melén et al., 1996). The longer isoform contains a nuclear localization signal (NLS)-like sequence in its first 25 amino acids and appears to localize preferentially to nuclear pores, whereas the 76 kDa form is cytoplasmic. Recent structural and evolutionary studies identified critical antiviral specificity determinants in MxA. A disordered loop, called L4 that is also present in MxB protrudes from the stalk (Figure 1B). An analysis of MxA orthologs from simian primates identified L4 as a target interface under positive selection and revealed that variations in L4 dictate antiviral specificity toward orthomyxoviruses (Mitchell et al., 2012). Additional "hot spots" of positive selection may represent alternative target specificity determinants.

Now, Liu et al. (2013) demonstrate that MxB, but not MxA, inhibits the replication of a well-known HIV-1 strain (NL4-3) in cell culture. Expression of MxB inhibited HIV-1 growth in a permissive CD4+ T cell line while depletion of endogenous MxB reduced the anti-HIV-1 effect of IFN-α in a human astroglioma cell line known to respond well to IFN treatment. These and additional experiments clearly demonstrate that MxB is responsible for a large part of the antiretroviral effect induced by type I IFNs. It was conceivable that MxB